

Figure S1. Growth of *C. difficile* is impacted with increases in alkaline pH in liquid culture. Strains 630 Δ erm (A) and R20291 (B) were cultivated in liquid 70:30 broth at a pH of 8.0, 8.2, 8.4, 8.7, and pH 8.9, respectively. Data were analyzed by one-way ANOVA and Dunnett's test compared to pH 8.0. * indicates P value of ≤ 0.05 ; ** indicates adjusted P value of ≤ 0.01 ; n=3.

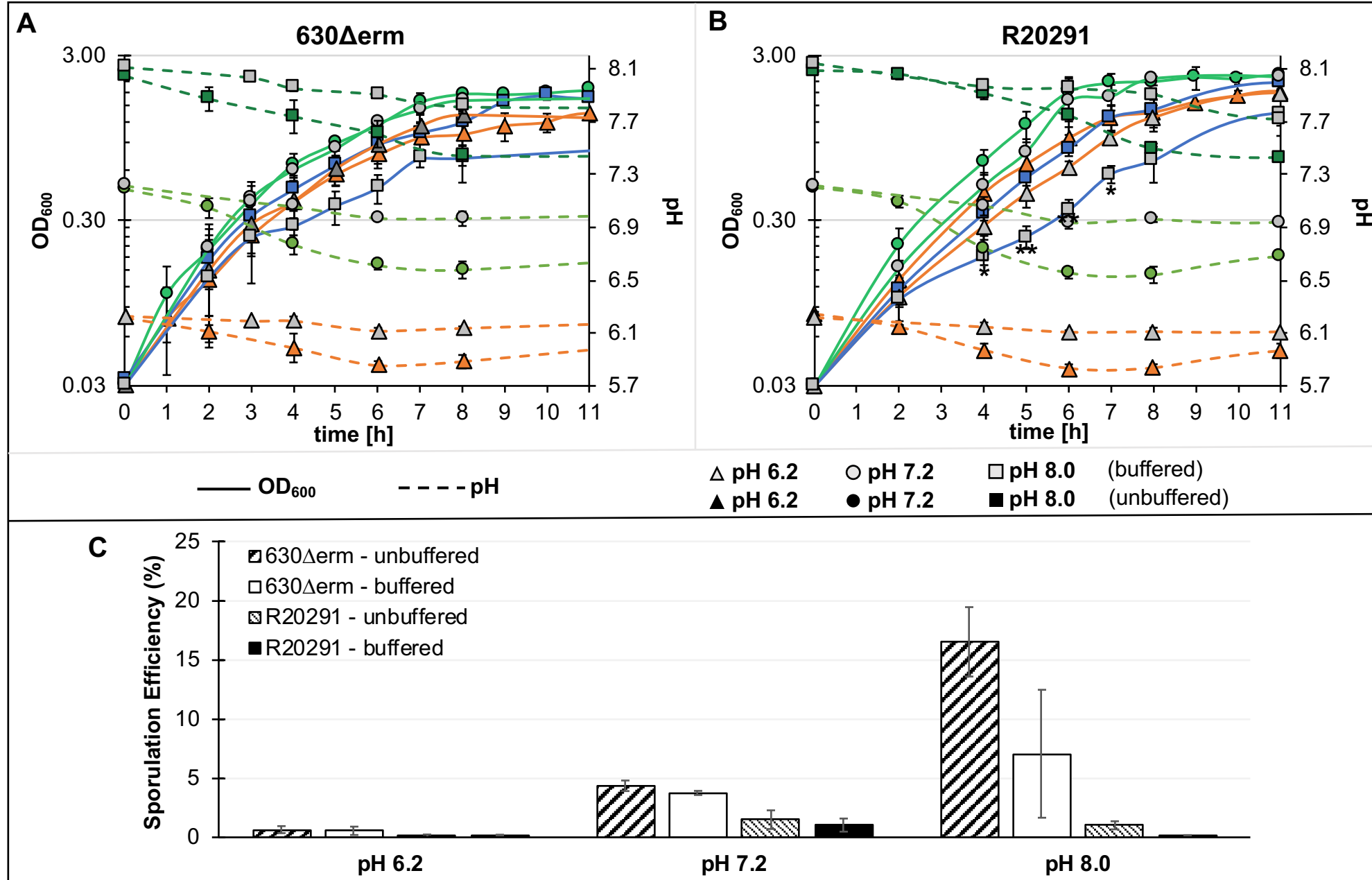


Figure S2. Growth and sporulation of *C. difficile* is similarly impacted by pH under buffered medium conditions. 630 Δ erm (A) and R20291 (B) were cultivated in 70:30 cultures at a pH of 6.2 with or without 0.1 M MES, or at pH 7.2 or 8.0 with or without 0.1 M HEPES, respectively. Only for R20291, a pronounced decrease in growth in buffered pH 8.0 culture compared to the unbuffered pH 8 culture was observed (* $P \leq 0.05$; ** $P \leq 0.01$). (C) Sporulation of strains under buffered medium conditions compared to non-buffered cultures. No statistical differences were observed. Data were analyzed by Student's two-tailed t -test. Experiments were performed three or more times.

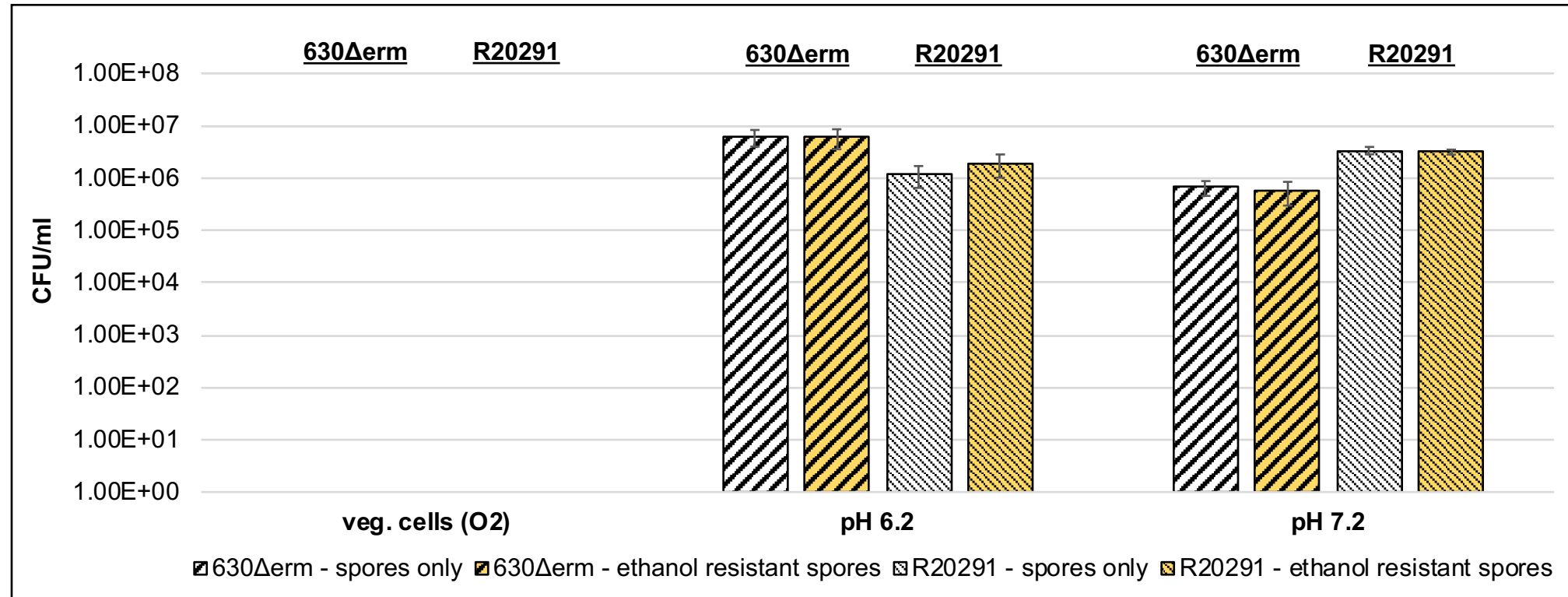


Figure S3. Spores of R20291 and 630Δerm generated at pH 6.2 and pH 7.2 in 70:30 cultures germinate and are ethanol-resistant. 24 h cultures at the respective pH were exposed to oxygen for >10 days to kill remaining vegetative cells (veg. cells O₂). After addition of 1% BSA, samples were analyzed for germination (spores only) and for ethanol-resistance (28.5%, 15 min) on BHIS + 0.1% TA. Most of the small, round spores of R20291 produced at pH 6.2 in liquid (see Figure 3), germinated and were ethanol-resistant. pH 7.2 spores were used as a control. The experiment was performed three independent times; n=3.

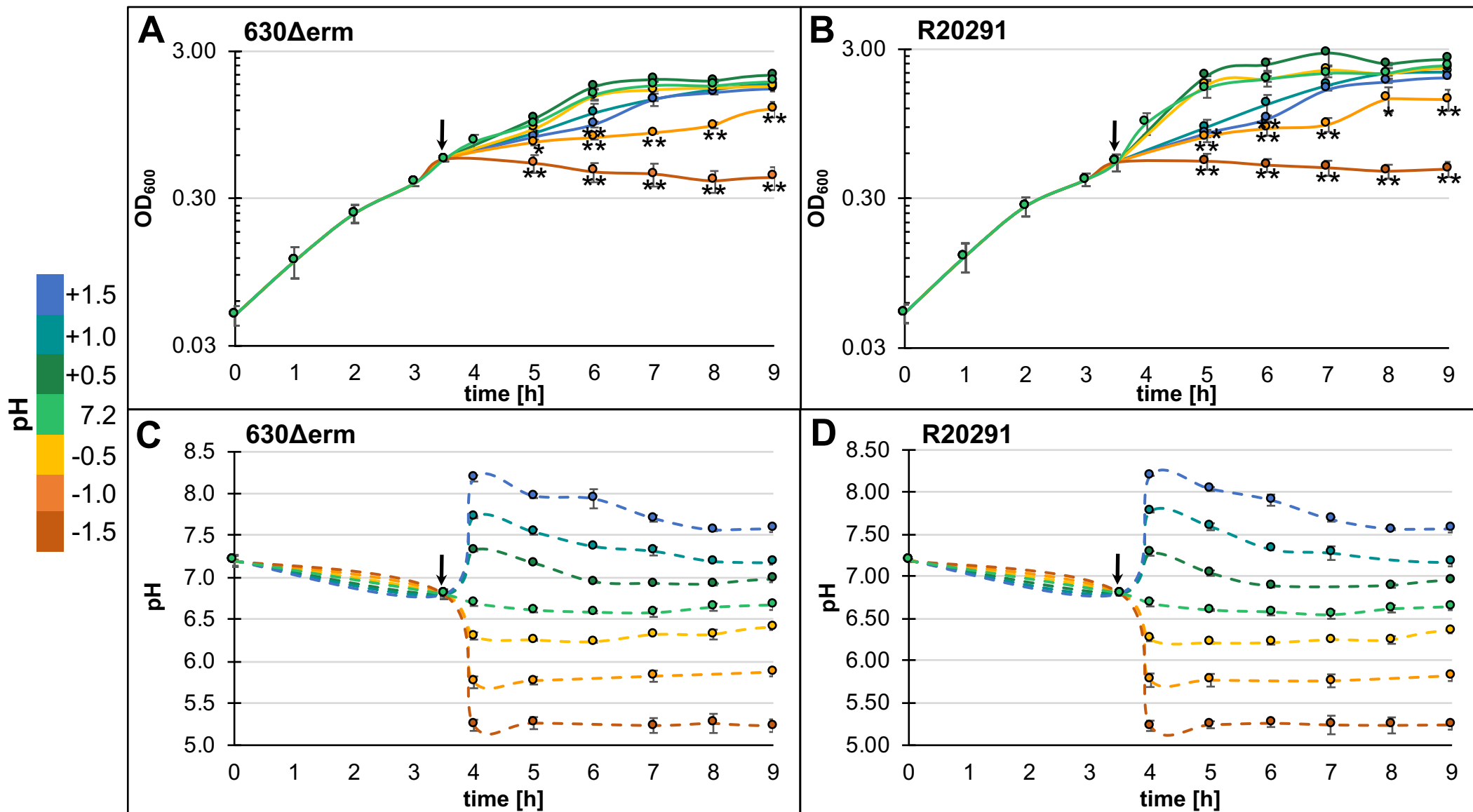


Figure S4. A rapid change in pH influenced growth of *C. difficile* in liquid culture. Strains 630 Δ erm (A,C) and R20291 (B,D) were cultivated in 70:30 broth at an initial pH of 7.2. At an OD₆₀₀ of 0.5 (indicated by arrow), the pH was increased or decreased 0.5, 1.0, or 1.5 pH units, respectively. A significant decrease in growth could be observed when the pH was rapidly shifted (+/-) 1.0 or -1.5 units. Data were analyzed by one-way ANOVA with Dunnett's test for multiple comparisons to growth at pH 7.2. * indicates P value of ≤ 0.05 ; ** indicates adjusted P value of ≤ 0.01 ; n=3.